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14. ABSTRACT
The primary purpose of this proposal was to demonstrate that the membrane disintegrin, ADAM-15 mediates the interaction (binding) between prostate tumor cells and vascular endothelium and that in this regard ADAM-15 would support the metastatic spread of human prostate cancer. The successful completion of one of the earliest aspects of this proposal has given substance to the hypothesis and confidence as we move ahead with more functional analysis of this mechanism. These results clearly justify continuing with the proposed studies and may ultimately justify ADAM15 as a direct therapeutic target for metastatic prostate cancer.

15. SUBJECT TERMS
Metalloproteinase, metastasis, angiogenesis

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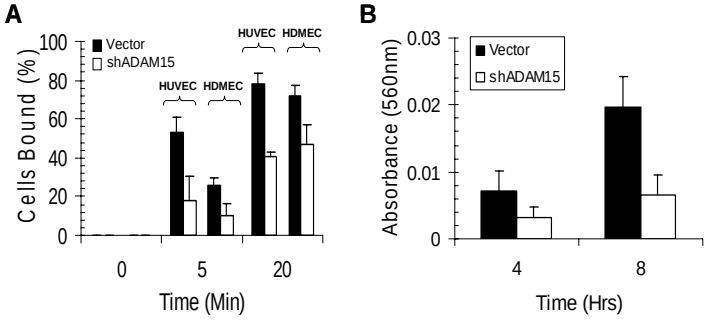
Table of Contents

INTRODUCTION: Several protease families including the ADAMs have been implicated in the regulation of angiogenesis. It is thought that ADAMs proteolytically process some of the key signaling components of inflammation and angiogenesis as well as adhesion molecules that comprise endothelial adherens junctions including vascular endothelial (VE)-cadherin, P-CAM-1, and integrins. VE-cadherin, the endothelial-specific cadherin, is known to regulate vascular integrity, endothelial cell migration, capillary tube formation and, as well as regulate activity of the VEGF receptor VEGFR-2 activity. A novel role for ADAM15 in endothelial adhesion was suggested by the observation that the distribution of ADAM15 in endothelial cell junctions appeared driven by VE-cadherin. This proposal is based on recent studies by this laboratory using human tissue, serum and cell lines demonstrating increased expression of the membrane disintegrin, ADAM15 in metastatic prostate cancer. We hypothesize that aberrant ADAM15 function mediates interactions between tumor cell and the vascular endothelium promoting angiogenesis and intravazation during prostate tumor progression. The primary goals of this proposal will attempt to delineate the specific function of ADAM15 in (i) human prostate tumor cell interactions with human endothelium and effects on angiogenesis and intravazation (ii) characterize the action of three key functional motifs of ADAM15 in prostate tumor angiogenesis and intravazation and (iii) identify the catalytic substrate(s) of ADAM15 that mediate the malignant activities of this protein. To initiate these studies of ADAM15 in angiogenesis of human blood vessels in vivo, we have successfully shown that ADAM15 functions in tumor cell/endothelial cell interactions in Trans-endothelial Migration Assays (Figure 1).

BODY: In order for cancer cells to metastasize, they must interact with the surrounding microvasculature and intravasate through the vascular endothelium to gain access to the blood stream. To assess the potential role of ADAM15 in tumor cell-endothelial interaction, we performed a tumor cell-endothelial cell adhesion assay. Vector or shADAM15 PC-3^{Luc} cells were plated on two types of primary human endothelial cell (HUVEC or HDMEC) monolayers for 0, 5, and 20 minutes and bound cells calculated as described in the materials and methods. We observed that shADAM15 PC-3^{Luc} cells had a significantly reduced ability to adhere to the endothelial cell monolayers in comparison to the vector control (Figure 1A). Both vector and shADAM15 epithelial cells attached more effectively on HUVEC monolayer than the HDMEC monolayer. To assess whether ADAM15 reduction would also affect epithelial cell intravasation *in vitro*, we performed a transendothelial migration (TEM) assay. Vector or shADAM15 PC-3^{Luc} cells were seeded on top of a confluent HUVEC monolayer in a transwell chamber and stimulated for 4 or 8 hours. Nearly 3-fold fewer shADAM15 PC-3^{Luc} cells were found to have migrated through the endothelial monolayer at the 8 hour time point in comparison to the vector control (Figure 1B). This data strongly supports a functional role for ADAM15 in prostate tumor cell interaction with vascular endothelium and the metastatic progression of human prostate cancer.



A) Vector or shADAM15 PC-3^{Luc} cells were incubated on top of confluent HUVEC or HDMEC monolayers and bound cells counted at indicated times. B) Transendothelial migration of vector or shADAM15 PC-3^{Luc} cells through a confluent endothelial monolayer at the indicated times.

ACCOMPLISHMENTS:

1. We have confirmed that ADAM15 knockdown reduces interactions with vascular endothelial cells and trans-endothelial migration. This completes task 1b.

2. We have completed the first experiments with respect to Task 1a to evaluate the role of ADAM15 in angiogenesis of human blood vessels in vivo using the SCID mouse model of human angiogenesis. As stated in the proposal this study was done in collaboration with the laboratory of Dr. Jaques Nor. Microvessel density was evaluated by immunohistochemistry using anti-human CD31 and anti-human Factor VIII antibodies which are specific markers of human vascular endothelium. Unfortunately, the ADAM15 knockdown is so efficient in inhibiting tumor growth in the PLLA scaffolds that very little tumor forms with which to make the analysis. In conclusion, there is a marked reduction in vascular endothelial markers in this model. What we cannot discern yet is if this is due to specific attenuation of angiogenesis or due to reduced growth of these tumors. We are currently thinking about alternative approaches to delineate.

3. With respect to task 2a the metalloproteinase-dead and the disintegrin-dead mutant constructs have been completed and cell transfection studies will start soon. The Src homology-dead mutation of ADAM15 is still be worked out as it has exhibited some difficulties.

REPORTABLE OUTCOMES:
We have completed task 1 and have initiated task 2a. Two functional mutations for task 2 have been completed. Abdo Najy who was a graduate student in my lab received a DOD pre-doctoral fellowship that covered his salary and tuition through complementing some of this work. We have published a manuscript in the journal Cancer research which describes the endothelial assay results in figure 5. The DOD is cited as the funding source. We have one manuscript under review describing the function of ADAM15 in the cleavage of E-cadherin human prostate cancer.

CONCLUSION: Our early findings in this project do indeed indicate that ADAM15 regulates prostate tumor cell/endothelial interactions. The structure function studies and mutations studies will validate the structural and modular requirements for these interactions.

BIBLIOGRAPHY:
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